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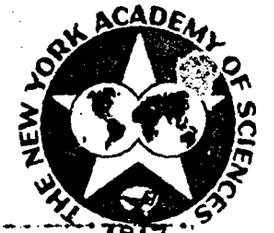

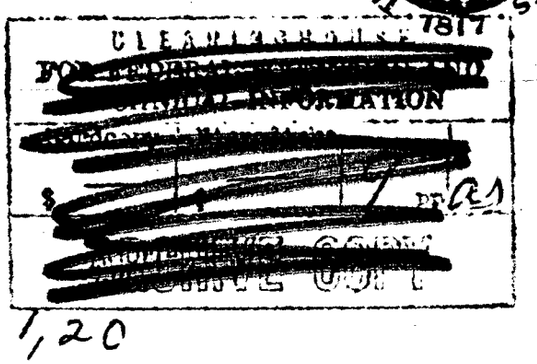

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THE PARTICHROME ANALYZER FOR THE DETECTION AND ENUMERATION OF BACTERIA

Sol S. Nelson, Orvil E. A. Bolduan*
United States Army Chemical Corps, Fort Detrick, Frederick, Md.

William A. Shurcliff†
Polaroid Corporation, Cambridge, Mass.

Introduction

The problem of detecting and counting aerosolized bacteria has been approached in several ways. One of the simplest methods is to collect a sample and examine it microscopically, using a standard bacteriological stain. The automation of such a device requires a processing system to collect and stain the sample and a scanner to recognize the stained organism against a background of unstained particles.

The instrument reported here differs from previously described devices for scanning microscopic fields in that instead of responding to particle size or total numbers it responds to color.

In our laboratory, the instrument has been applied to the detection and counting of aerosolized bacteria without regard to the specific identification of the organism. However, the device lends itself to the detection and counting of any microscopic particles that have a characteristic spectral absorption or that are dyed by a substance having such a characteristic color.

It is proposed to name the instrument the "Partichrome," a loose combination of syllables that indicate the factors "particle" and "color."

The Instrument

The instrument is made up of three principle components: the processor, the scanner, and the signal analyzer. Although the components are integrated into a single device, they are constructed so that each can be modified for a particular application.

The processor. The processor collects a sample on a reel of Cronar‡ tape, applies a suitable dye, and then moves the tape into the scanner.

In selecting a method of sample collection, consideration was given to electrostatic precipitation, thermal precipitation, and impaction. Some of the factors that affected evaluation of these methods were sample size, elimination of particles whose size precluded the possibility that they were bacteria, and power requirements. The method of impaction was selected because of its simplicity and its high efficiency. The technique is implemented by an impactor patterned after the fourth stage of the Casella impactor. Air is drawn in at a rate of 17.5 l. per min., and the particles are deposited with an efficiency of greater than 90 per cent if the surface is first coated with a thin film of high-viscosity immersion oil. In order to reduce the deposition of large parti-

* Present address: United States Army Research and Development Liaison Group, New York, N.Y.
† Present address: Cambridge Electron Accelerator, Cambridge, Mass.
‡ Obtained from E. I. du Pont de Nemours and Company, Inc., Wilmington, Del.

cles, a cyclone-type separator is placed ahead of the impactor. This separator is designed to prevent the impaction of 90 per cent of particles larger than 5 μ.

After experimenting with 65 visible dyes, ethyl violet was selected for the staining operation because it was found to be most suitable for general bacterial staining and, as observed empirically, very little of the "dust" normally found in the air took up the dye.

The staining procedure carried out by the processor is as follows: (1) HCl at 60° C. for 1 min. (1.9 per cent aqueous solution); (2) water rinse; (3) ethyl

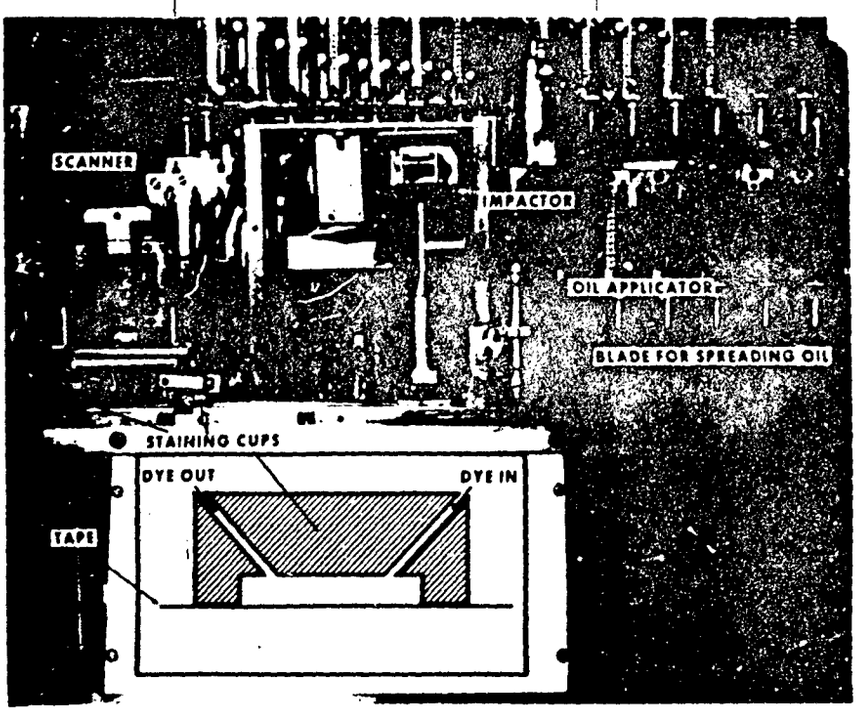

FIGURE 1. Processor.

violet (1 per cent in 0.5 per cent aqueous solution of Triton X-100, 85° C., 45 sec.); (4) water rinse; (5) dry; (6) nitrobenzene, 5 sec.; (7) rinse in xylene. The nitrobenzene in step 6 of the staining procedure is thought to remove dye from nonbiological materials. Omission of this step results in a higher background than is obtained with the staining procedure as described above.

The processor* shown in FIGURE 1 accommodates a 5-inch reel of tape which is threaded into a take-up reel in the scanner. As the tape moves past the oil applicator, a thin film of immersion oil is applied. The tape stops under the impactor where the sample is deposited for a predetermined time (60 sec. in our laboratory). The tape then proceeds to the staining stations (while the

* Designed and constructed by Photomechanisms, Inc., Huntington Station, N.Y.

sample is being heat-fixed) where the dye solutions are applied. The application of the dye is accomplished by means of a Kel-F staining cup shown in the insert in FIGURE 1. The stations are designed so that an additional cup may be installed if the dyeing procedure and programming make it desirable; also, a cup may be deleted when necessary. Adjustable timers were included to allow for experimentation with the staining technique. They are useful for such purposes, but are unnecessary for a procedure whose timing is fixed. Where the instrument is to be used for a variety of staining procedures, the adjustable timers can be replaced by a simple cam system.

The scanner. The optical system of the scanner is shown schematically in FIGURE 2. Light from a zirconium arc lamp passes through a pinhole. The beam of light is then displaced about 1 cm. off-axis by a system of 2 mirrors and a prism. The displaced beam then passes through an oil-immersion objective that minifies the beam and focuses it on the specimen slide.

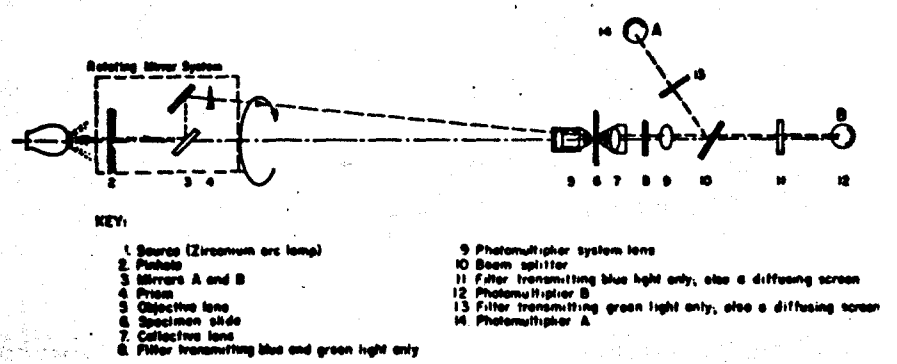

FIGURE 2. Optical system of the scanner.

Beyond the slide the light passes through a condenser to a beam splitter. Each beam is then directed toward a 1P28 phototube that is made sensitive to the desired color by a suitable filter placed in front of it. For use with ethyl violet, one phototube is made sensitive to blue light by means of a blue filter with a maximum transmission at 470 mμ and the other is made sensitive to green by a filter that transmits at about 600 mμ.

The scanning pattern is obtained by rotation of the mirror system and simultaneous translation of the slide transverse to the optical axis with a slow, uniform motion. The scanning pattern obtained in this manner is shown in FIGURE 3. Thus when white light passes through an unstained (opaque) particle, both the blue and green components are absorbed and the output of both phototubes is attenuated. When the beam of light passes through a blue-stained particle, the green component of the light is absorbed to a greater extent than the blue, and the output of the green phototube is attenuated to a greater degree than that of the blue phototube. In this manner, blue particles may be counted in the presence of other particles.

The signal analyzer that receives pulses from the photomultiplier consists of a base-line straightener, amplifier, discriminator, and anticoincidence circuit.

Calculated performance. The performance expected from the instrument may be estimated from a knowledge of the design parameters. Thus, for the instrument in use in our laboratory, the relationship between scan count and concentration of the aerosolized bacteria may be estimated from the following: r, scanning speed (20 rev./sec.); d, scan diameter (100 μ); w, width of light beam (1.5 μ); dimension of impactor jet (14 mm. × 0.27 mm.); a, area of impactor jet (0.0378 cm.²); f, impaction flow rate (17.5 l./min.); t_p, time of scan by instrument (sec.); t_s, time of collection (minutes); N, number of

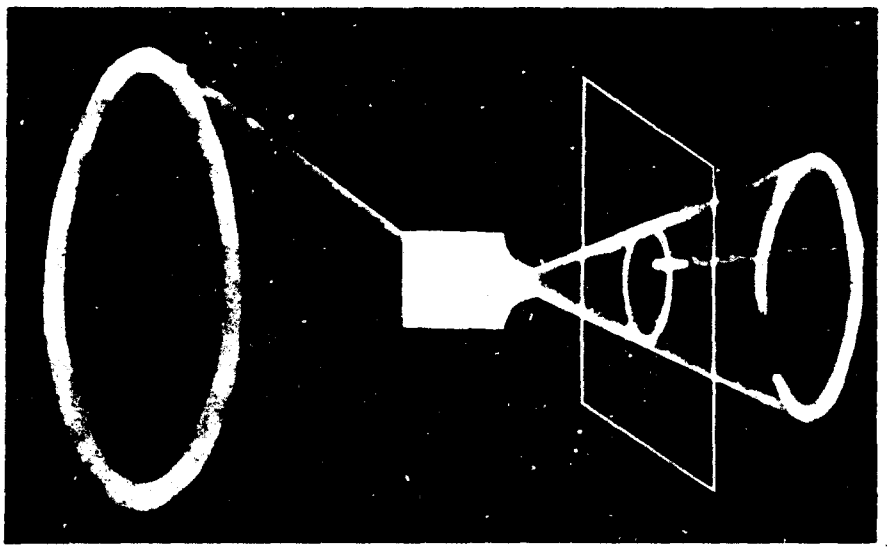

FIGURE 3. Scanning principle of the bacterial detector, showing scanning pattern obtained.

counts recorded in time t_p; S, surface concentration of particles (particles/cm.²); C, concentration of particles in aerosol (particles/l. of aerosol); and

$$S = \frac{N}{\pi\, dwrt_p} = \frac{1.06 \times 10^4 N}{t_p}. \qquad (1)$$

The following assumptions are made:

(1) The surface concentration directly under the center of the jet (the section scanned) is the same as the concentration that would be obtained if all the material were deposited on the surface in an area having the same dimensions as the jet itself.

(2) The impaction efficiency is 1.0.

(3) The particles are uniformly distributed.

Then

$$C = Sa\frac{1}{f}\frac{1}{t_s} = \frac{1.06 \times 10^4 N}{t_p}(.0378)\left(\frac{1}{17.5}\right)\left(\frac{1}{t_s}\right) \qquad (2)$$

Thus, when t_p = 80 sec. and t_s = 1 min.,

$$C = \frac{N}{3.5}. \qquad (3)$$

Results

In evaluating the performance of the Partichrome, several parameters may be measured independently. Such measurements are aimed at testing the

TABLE 1
INCIDENCE OF "FALSE" TO "TOTAL" COUNTS WHEN SCANNING DUST THAT HAS *NOT* BEEN SUBJECTED TO THE STANDARD STAINING PROCESS

Per cent of false counts (blue count/total count × 100)	No. of slides	Per cent of slides
0.00	21	53.9
>0.01 to 0.02	7	18.0
>0.02 to 0.05	5	12.8
>0.05 to 0.10	3	7.7
>0.10 to 0.20	1	2.5
>0.20	2	5.1
Total	39	100.0

TABLE 2
INCIDENCE OF "FALSE" TO "TOTAL" COUNTS WHEN SCANNING DUST THAT *HAS* BEEN SUBJECTED TO THE STANDARD STAINING PROCESS

Per cent false counts (blue count/total count × 100)	No. of slides	Per cent of slides
0.00 to 0.02	5	17
>0.02 to 0.05	8	27
>0.05 to 0.10	6	20
>0.10 to 0.15	8	27
>0.15 to 0.20	2	7
>0.20	1	3
Total	30	101

reliability of color discrimination by the optical system and the reliability of staining and scanning procedures.

Carbon particles. A test of the reliability of the scanner is to measure the signals obtained from particles that are known to absorb both the blue and green components of light. Experiments with 1-μ carbon particles show that less than 0.1 per cent of the total particles scanned count as blue particles. The significance of this measurement is that the optical system of the scanner is capable of recognizing that a particle is absorbing both blue and green components of the light.

"Dust" counts. Experiments were carried out to determine the incidence of "false" blue counts, *i.e.*, the number of particles normally suspended in the atmosphere that will be counted as blue particles by the scanner. The results

shown in TABLE 1 indicate that this false count is essentially less than 0.2 per cent when no stain is applied. The results shown in TABLE 2 indicate that the false count obtained after applying the dye is substantially less than 2 per cent.

Bacterial counts. To determine the reliability of the Partichrome in counting aerosolized bacteria, aerosols of various concentrations of spores of *Bacillus subtilis* var. *niger* were sampled and stained. Visual microscopic counts were then made, and the aerosol concentration, A_v, was calculated from these counts. The ratio of measured concentration to the concentration calculated by means of Equation 3, C/A_v, was computed for each determination. For a

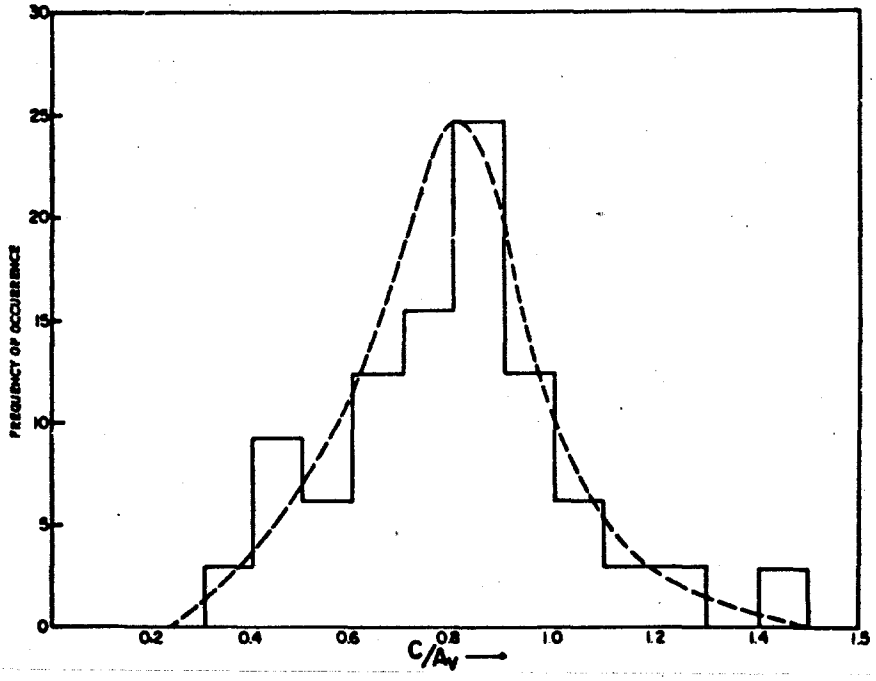

FIGURE 4. Frequency plot of the ratio C/A_v.

total of 32 determinations with aerosol concentrations ranging from 400 to 6000 particles per l., the average value of C/A_v was 0.80 with a standard deviation of 0.25. A frequency plot of the ratio C/A_v is shown in FIGURE 4.

Discussion

Systems for optical scanning of a specimen on the stage of a microscope fall into two broad categories:

Flying spot systems. In these systems the optical beam, minified by the objective lens, travels across the surface of the specimen. The travel may be accomplished by: (1) a moving miniature lamp; (2) a cathode ray tube whose luminous spot moves;[1] (3) a rotating Nipkov disk with the pinholes arranged on a spiral path of constantly decreasing radius or on a circular path; (4) a

single pinhole with rotating periscope (the principle used for the scanner described here); (5) a single pinhole with traveling microscope objective; (6) a fixed spot with two-dimensional movement of the specimen.

Image-space systems. In these systems the optical beam is broad and remains stationary with respect to the specimen. The scanning motion occurs *beyond* the specimen, *i.e.*, in image space. The motion may employ: (1) a miniature moving detector; (2) a TV camera tube;[2] (3) a rotating Nipkov disk,[3] rotating periscope or traveling collective lens.

The rotating periscope system seemed to be most suitable for our application in view of the following considerations:

(1) The use of the same luminous spot on the lamp makes it possible to avoid the fluctuations due to differences in light intensity from different spots on the lamp.

(2) The continuous rotation avoids discontinuities that are inevitable with the Nipkov disk.

(3) The use of the rotating periscope circumvents the difficulty of making all the holes in the scanning disk of uniform diameter, or employing a system that corrects for differences in hole sizes.

The anticoincidence signal analyzer was compared experimentally with a circuit in which the blue signal was inverted and then added to the green signal. Both types of signal handling were effective, but the anticoincidence circuit allowed for a greater tolerance in focus. It is postulated that this is due to the fact that if the pulses from the two phototubes are not in phase, the adder circuit will give false blue counts. The anticoincidence circuit, on the other hand, will function so long as there is any overlap at all of the two pulses.

The results obtained with the instrument indicate that a fairly reliable count of stained bacteria may be obtained. In the case of its application to the study of aerosolized bacteria, a fairly reliable estimate of bacterial concentration may similarly be obtained. The performance of the instrument is especially interesting in view of the fact that some obvious limitations are inherent in the design:

(1) The circular scanning system, chosen primarily for mechanical stability and convenience, results in considerable overlap and redundant counting along the edge of the 100-μ diameter scanning path whose tangent is parallel to the direction of slide translation. At the same time, areas are missed along the edge of the scanning path where tangent is normal to the direction of slide translation. However, if the particles are uniform and randomly distributed, this deficiency does not introduce significant error.

(2) No provision is made for preventing multiple counting. Such provision could be made either by use of a double spot[1] or by a memory system[4] that correlates the results from adjacent scanning lines. With such a system, a count is registered only when a signal is registered at a position in a line where no signal was registered for the previous scanning line. Should this refinement be required, the system can be incorporated into the present instrument.

(3) The assumption that the impacted area is the same as that of the jet, and that the particles are uniformly deposited is not strictly valid. However,

the close agreement between the calculated performance and the observed performance indicates that it is a reasonable assumption to a first approximation.

Acknowledgments

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